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SHORT COMMUNICATION

Potential of a synthetic aggregation pheromone for integrated pest management of Colorado potato beetle

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Abstract 1 The relative number of colonizing adult Colorado potato beetles (CPB) *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) coming to pitfall traps baited with the aggregation pheromone (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I] and the use of the pheromone in a trap crop pest management strategy were evaluated in the field for the first time.

2 More than five-fold more adult *L. decemlineata* were caught in pitfall traps baited with the pheromone compared with controls. However, attraction to the pheromone diminished after 5 days in the field.

3 In the trap crop management strategy, more colonizing adults were present in pheromone-treated rows of potatoes compared with untreated middle rows.

4 Significantly fewer *L. decemlineata* egg masses and larvae were found in potato plots that were bordered by pheromone-treated rows, or bordered by imidacloprid + pheromone-treated rows, or rows treated at-planting with imidacloprid compared with untreated (control) potato plots.

5 Densities of *L. decemlineata* egg masses and larvae and percentage defoliation were significantly lower, and marketable tuber yield significantly higher, in conventional imidacloprid-treated potatoes compared with all other treatments.

6 Although our results demonstrate the potential for use of the aggregation pheromone in the management of *L. decemlineata* in the field, more research is needed to optimize the release rates of the attractant and incorporate control methods for cohabiting pests.

Keywords Aggregation pheromone, Colorado potato beetle, integrated pest management, trap crop.

Introduction

Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is one of the most important insect pests of potato, *Solanum tuberosum* L., in the U.S.A. and Europe. Larvae and adults feed on leaves and can quickly defoliate a plant and affect tuber yield. In the U.S.A., the insect has developed resistance to more than 25 insecticides (Roush *et al.*, 1990; Ioannidis *et al.*, 1991; Grafius, 1997), which left many potato growers with very few options for control in the 1980s. Currently, potato growers have several effective chemicals to combat CPB, including the neonicotinoid compounds, imidacloprid and thiamethoxam, and the fermentation metabolites, spinosad and avermectin (Linduska *et al.*, 2000; Kuhar & Speese, 2002; Kuhar *et al.*, 2003a). However, these products are expensive and overused, particularly imidacloprid, which is a systemic

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insecticide typically applied at planting as a prophylactic control measure. Moreover, resistance to imidacloprid has already been found in numerous *L. decemlineata* populations in the U.S.A. (Olson *et al*., 2000; Zhao *et al*., 2000), and will probably spread.

From the standpoints of environmental stewardship, insecticide resistance management and economic savings to potato growers, it is important that we seek methods to reduce the use of insecticides for the control of CPB in potatoes (Casagrande, 1987; Hare, 1990). An effective beetle attractant could help to reduce insecticide usage by: (i) improving sampling methods used to detect colonizing beetles for decision-making in integrated pest management (IPM); (ii) incorporating the attractant into a trap crop to draw beetles away from unprotected crops into smaller areas where they can be controlled; or (iii) using the attractant as a component of an attracticide formulation, which would increase the specificity of a control agent such as a insecticide, thus decreasing the negative effects on other organisms within the potato ecosystem.

Synthetic attractants have been discovered for CPB. Kairomonal blends comprised of two or three plant volatiles were attractive to both sexes of CPB (Dickens, 2000). A three-component blend consisting of (Z)-3-hexenyl acetate, (±)-linalool, and methyl salicylate attracted both larvae and adults (Dickens, 2002). More recently, a male-produced aggregation pheromone, (*S*)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol [(*S*)-CPB I], was found that is highly attractive to *L. decemlineata* adults (Dickens *et al*., 2002). An economically feasible synthesis has been devised for (*S*)-CPB I, and quantities were synthesized for use in the field (Mori & Tashiro, 2004).

In the present study, we present the first field evaluations of the CPB aggregation pheromone, (*S*)-CPB I, for use in sampling colonizing CPB adults with pitfall traps and in a trap crop management strategy in potatoes.

Materials and methods

Chemical lures

(*S*)-CPB I was 96.5% optically pure (Mori & Tashiro, 2004). The pheromone was released from red rubber septa obtained from Fisher Scientific Co., Cat. no. 03-215-5 (Fisher Scientific International Inc., Hampton, New Hampshire). Prior to treatment, septa were washed several times in hexane. Individual septa were then treated with 100 μL of a 50 μg/μL solution in hexane or 50% hexane : 50% ethyl acetate to give 500 μg of pheromone per septum. The solvent was allowed to evaporate prior to wrapping each septum in aluminium foil and placing them in plastic freezer bags in an ice chest for transport to the field.

The rate of release of (*S*)-CPB I from the rubber septa were determined using an automated volatile collection system (Analytical Research Systems, Inc., Gainesville, Florida) modified from one previously described by Heath & Manukian (1994), and as described in detail elsewhere (Dickens *et al*., 2002). In brief, a single rubber septum containing the pheromone was placed in a 5-L volatile collection chamber from which volatiles were collected for eight 6-h periods or eight 12-h periods by programming the switching of eight ports of a manifold holding volatile collection traps containing SuperQ adsorbent (Alltech Industries, Deerfield, Illinois). This procedure was replicated three times for the 6-h collections over 48 h in the laboratory at ambient conditions. Volatiles were extracted from each trap with 100 μL of hexane, of which 50 μL were collected in 300 μL cone vials. N-decane (10 ng) was added to each sample as an internal standard. One-microliter samples were injected into a Hewlett Packard™ Model 5890A (Hewlett Packard, Palo Alto, California) gas chromatograph (GC) equipped with an HP-5 capillary column (crosslinked 5% PH ME siloxane; film thickness 0.25 μm; length 30 m; inner diameter 0.25 mm) and a flame ionization detector. The GC was programmed to hold an initial temperature of 50 °C for 2 min after injection, increase 15 °C/min to 235 °C, and hold for 8 min. The mean release rate for the pheromone was determined for the 16 6-h periods. Hourly release rates were approximated based on these data.

Pitfall trap study

In June 2004, we conducted pitfall trap experiments at the Virginia Tech Eastern Shore Agricultural Research and Extension Center near Painter, Virginia. A 0.5-ha section of land was cultivated to remove weeds and harrowed to create a relatively uniform ground surface. We arranged 10 separate pitfall trap stations around a circle in the field of diameter 30.5 m so that each station was 15.2 m from the centre 'release' point and 9.1 m from the next closest trap. Each pitfall trap station consisted of two pitfall cups (diameter 10.2 cm) buried flush to the ground and containing ethylene glycol as a killing agent for insects falling into the trap. A wooden stake was placed between the two cups to mark the trap location and to hold the pheromone lure 40 cm above the ground. Traps were numbered consecutively 1–10 around the circumference. On 2 June, we fastened a rubber septum containing 500 μg of (*S*)-CPB-I to the wooden stake using a binder clip at each odd-numbered location. The untreated controls had a stake and binder clip with no rubber septum.

Immediately after installation of the pheromone, we collected 400 CPB adults from a nearby potato field and released them in the centre of the pitfall trap area. At 24 and 48 h after release, we assessed the catch of CPB adults in the traps. On 7 June, using the same pheromone-treated and control septa that were placed in the field previously, we re-randomized the pitfall trap locations and replicated the experiment with another set of 400 field-collected adults. Again, we assessed beetle catch at 24 and 48 h. We analysed the effect of (*S*)-CPB-I on *L. decemlineata* pitfall trap catch using Student's *t*-test.

Trap crop study

We planted field plots of 'Superior' potatoes on 24 March 2004 at the Virginia Tech Eastern Shore Agricultural Research and

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Extension Center near Painter, Virginia. The experiment consisted of four treatments arranged in a randomized complete block with four replications. Individual plots consisted of eight rows of potatoes × 7.6 m long (55.5 m^2). Plots were spaced approximately 9 m apart. Plants were spaced approximately 30 cm apart within a row. Treatments included: (i) a 'conventional insecticide' in which all eight rows were treated at-planting with an in-furrow application of imidacloprid at a rate of 0.20 lb active ingredient (ai)/acre; (ii) a 'pheromone border' in which the middle four rows were untreated and the outer two rows contained CPB-I pheromone lures; (iii) a 'pheromone + insecticide border' in which the middle four rows were untreated and the outer two rows were treated with imidacloprid as described previously and contained pheromone lures; and (iv) an 'untreated control' in which all eight rows were left untreated.

We installed septa treated with (*S*)-CPB I in the designated plots on 14 May 2004 soon after overwintering adult CPB commenced activity. We fastened septa with a paper clip to every other plant in the outer two rows of the plot (approximately 80 septa per plot). One septum was hung at approximately ground level; the other was hung from the highest stem on the plant.

On 15, 17, 21, 27 May and 3 June, we counted the number of CPB egg masses, larvae and adults on 10 plants in the middle rows of each plot and 10 plants in the border rows. On 3 June, we visually estimated the percentage defoliation in the plots. On 9 July, we harvested the middle two rows of each plot with a single-row mechanical potato harvester and evaluated tuber yield according to U.S. standards (Grade B, small A, large A and Chef).

To evaluate the effect of the pheromone on CPB adult colonization on potatoes, we analysed the numbers of adults over time between pheromone-treated and untreated rows using repeated measures analysis of variance (ANOVA) procedures. To evaluate the effect of CPB pheromone as a pest management trap crop tool, we analysed the effect of treatment on the peak density of each *L. decemlineata* life stage, percentage defoliation and marketable tuber yield of the middle rows of each potato plot using ANOVA procedures. If required to meet conditions of normality, data were square-root transformed prior to analysis. Percentage defoliation data were arc-sine, square-root transformed prior to analysis. All means were separated using Fisher's protected least significant difference at the $P < 0.05$ level of significance.

Results

Release rate of (*S*)-CPB I from lures

Over 48 h under laboratory conditions (*S*)-CPB I was released from rubber septa at a rate of 280 ng/6-h collection period (SE ± 34), an average of 46.7 ng/h.

Pitfall trap study

After 48 h, almost five-fold as many adult CPB were caught in traps baited with (*S*)-CPB I compared with nonbaited control traps ($t = 2.24$; d.f. = 4; $P < 0.0176$) (Fig. 1). After exposure of the lures for 5 days, fewer CPB were captured in the traps and the treatments were not significantly different.

Trap crop study

Densities of CPB adults, egg masses and larvae in each of the treatment plots over time are shown in Fig. 2. There was a significant treatment by date interaction on the number of CPB adults found on pheromone-treated border rows vs. untreated middle rows of potatoes ($F = 5.10$; d.f. = 3, 18; $P = 0.0099$). More adults occurred on pheromone-treated border rows than on untreated middle rows of the same plots on 15 May ($t = 5.14$; d.f. = 3; $P = 0.0143$) and 17 May ($t = 12.66$; d.f. = 3; $P = 0.0011$), which was 24 and 72 h, respectively, after pheromones were applied. Adult CPB densities in fields were relatively low after 17 May and were subsequently not different between pheromone-treated and untreated potatoes.

Cumulative densities of CPB life stages occurring on the middle four rows of all potato plots, as well as the border rows of pheromone-treated plots, were pooled over the sample dates (from 15 May to 3 June) and compared among treatments (Table 1). There was no significant treatment effect on the number of adults ($F = 1.82$; d.f. = 3, 9; $P = 0.2135$), but there were differences among treatments in numbers of egg masses ($F = 7.69$; d.f. = 3, 9; $P < 0.0075$) and larvae ($F = 20.85$; d.f. = 3, 9; $P < 0.0002$). Untreated control plots had significantly more egg masses than those protected by pheromone-treated borders or by conventional imidacloprid treatment. Conventional imidacloprid-treated plots had the fewest egg masses. Untreated control plots had significantly more larvae than all other treatments, and conventional

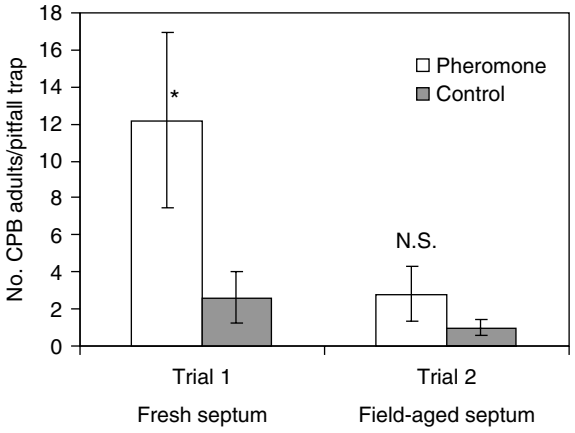

Figure 1 Catch of adult *Leptinotarsa decemlineata* in (*S*)-CPB I-baited and unbaited-control pitfall traps 48 h after approximately 400 newly eclosed adults were released in the middle of a field plot. Note the decrease in response to pheromone after septa were aged in the field for 5 days.

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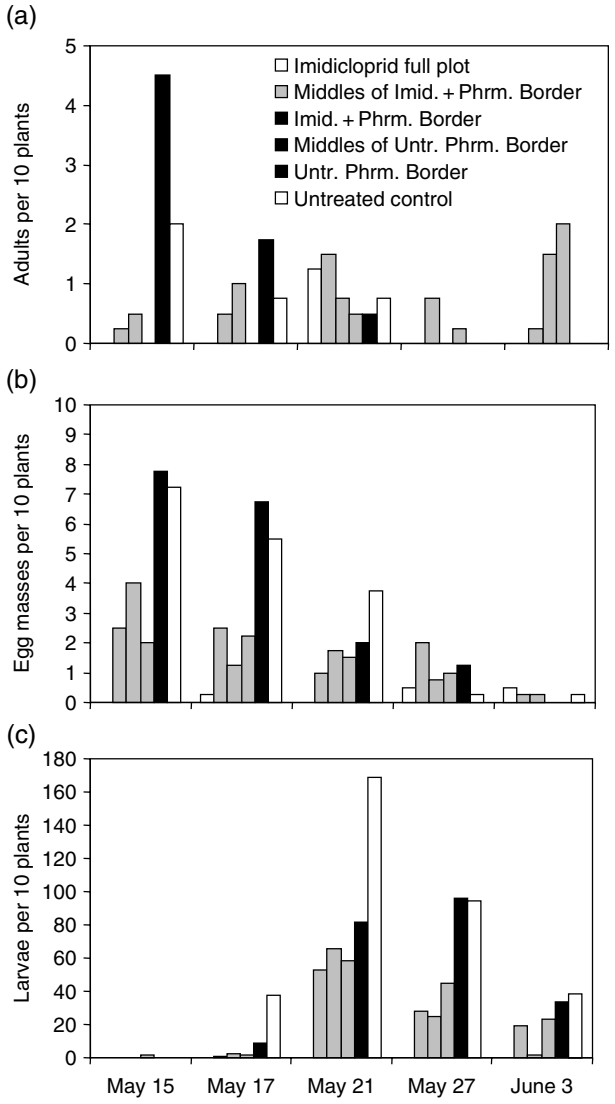

Figure 2 Numbers of (A) *Leptinotarsa decemlineata* adults, (B) egg masses and (C) larvae sampled over time on potatoes with various pest management strategies, Painter, Virginia, 2004.

imidacloprid-treated plots had the fewest larvae over all. There was a significant treatment effect on percentage defoliation ($F = 25.26$; d.f. = 3, 9; $P < 0.0001$) and marketable yield ($F = 15.61$; d.f. = 3, 9; $P < 0.0007$) with the conventional imidacloprid-treated plots having less defoliation and greater marketable yields than all other treatments (Table 1). It should be noted that relatively high densities (approximately two nymphs per leaf) of potato leafhoppers, *Empoasca fabae* (Harris), as well as 'hopperburn' injury to potato leaves, were evident in the pheromone trap crops and untreated control plots, but were absent in the imidacloprid-treated plots. Consequently, potato yield differences among the treatments were likely confounded by this pest in addition to CPB.

Discussion

The use of pheromones to draw beetles to a point source may greatly reduce the amount of insecticides necessary for effective control of CPB in potato fields. The present study demonstrated that the behaviour of postdiapausing adult CPB in the field can be manipulated with the use of the aggregation pheromone (Dickens *et al*., 2002). Almost five-fold as many adult CPB were captured in traps baited with (*S*)-CPB I than in unbaited control traps. Attraction to the pheromone-baited septa appeared to diminish after 5 days in the field. However, more research on pheromone release rates and the type of dispenser used could improve this situation.

Significantly more colonizing adults were observed on pheromone-treated border rows than on untreated middle rows of potatoes in the trap crop experiment. Furthermore, fewer egg masses and larvae were found on potato plots protected by pheromone-treated borders than untreated control plots. However, the pheromone-border trap crop was not as efficacious at controlling CPB as the conventional pesticide (imidacloprid) application over the entire plot. Potato tuber yield was significantly higher in the conventional imidacloprid-treated plots compared with the other treatments, but this difference was impacted not only by CPB, but also by potato leafhopper, which caused substantial 'hopperburn' in the pheromone trap crops and untreated control plots but not in the imidacloprid-treated plots. Kuhar *et al*. (2003b) showed that as few as one potato leafhopper nymph per leaf can significantly impact plant yield.

Martel *et al*. (2005) recently demonstrated a successful trap crop management strategy on CPB with the use of

Table 1 Mean ± SEM densities of *L. decemlineata* life stages, percentage defoliation, and marketable yield of potatoes produced with various pest management strategies for *L. decemlineata* in Painter, Virginia, 2004

			Cumulative numbers of *L. decemlineata* per 10 plants		
Treatment	Percentage defoliation	Marketable yield (cwt/acre)	Adults	Egg masses	Larvae
Conventionally managed plots[1]	1.3 ± 0.3[a]	1.3 ± 0.5[c]	0.0 ± 0.0[c]	2.5 ± 1.4[b]	156.2 ± 7.4[a]
Plots bordered by pheromone-treated rows	0.8 ± 0.3[a]	6.8 ± 1.9[b]	130.0 ± 26.6[b]	25.0 ± 2.0[a]	111.2 ± 11.4[b]
Plots bordered by imidacloprid + pheromone-treated rows	3.0 ± 1.2[a]	7.8 ± 2.6[ab]	103.5 ± 33.7[b]	22.5 ± 2.5[a]	104.1 ± 5.1[b]
Untreated control plots	3.5 ± 1.4[a]	17.3 ± 5.8[a]	344.3 ± 82.2[a]	38.8 ± 4.3[a]	97.5 ± 7.4[b]

[1]Conventionally managed plots were treated at-planting with an in-furrow application of imidacloprid at a rate of 0.20 lb ai/acre.

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the synthetic kairomone blend of (*Z*)-3-hexenyl acetate, (±)-linalool and methyl salicylate (Dickens, 2000, 2002). The synthetic pheromone is even more attractive than the kairomone blend (Dickens *et al*., 2002), and offers even greater potential for manipulating adult behaviour in the field. Clearly, additional research is needed on dispensing and optimizing the release rates of the pheromone in the field. Furthermore, because a combination of the pheromone and kairomone is preferred in laboratory bioassays over either treatment alone (Dickens, unpublished data), the combination of attractants should also be tested in the field.

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